http://www.icn.unal.edu.co/ Caldasia 33(1):55-66. 2011

PHYLOGENETIC RELATIONSHIPS AND REVIEW OF THE SPECIES OF *AURICULARIA* (FUNGI: BASIDIOMYCETES) IN COLOMBIA

Relaciones filogenéticas y revisión de las especies del género *Auricularia* (Fungi: Basidiomycetes) en Colombia

Andrés Felipe Montoya-Alvarez
Hiroshi Hayakawa
Yukio Minamya
Tatsuya Fukuda
Faculty of Agriculture, Kochi University, B200, Monobe, Nankoku, Kochi 783- 8502, Japan.
felipemontoyaa@gmail.com

Carlos Alberto López-Quintero
Ana Esperanza Franco-Molano
Laboratorio de Taxonomía y Ecología de Hongos, Instituto de Biología, Universidad de Antioquia, Apartado 1226, Medellín, Colombia.

ABSTRACT
The phylogenetic relationship of the species of the genus *Auricularia* and its allied taxa were investigated using the internal transcribed spacer (ITS) sequences of nuclear DNA. A molecular phylogenetic tree constructed using a total of 17 samples representing five species and two outgroups indicates that the species of *Auricularia* form a monophyletic group. Within the genus *Auricularia*, *A. mesenterica* is basal and the remaining *Auricularia* species form three clades; the first clade consists of *A. auricula-judae*; the second, of *A. fuscosuccinea*; and the third, of *A. polytricha.*

Key words. *Auricularia*, taxonomy, phylogeny, Colombia

RESUMEN
Las relaciones filogenéticas entre las especies del género *Auricularia* y especies cercanas se investigaron usando secuencias de ADN nuclear de la región (ITS). Se construyó un árbol filogenético molecular usando un total de 17 muestras, representando cinco especies, y dos grupos externos. Los resultados indican que las especies del género *Auricularia* conforman un grupo monofilético en donde *A. mesenterica*, se encuentra en la región basal del árbol y las otras especies estudiadas se ubicaron en tres clados. En el primer clado se ubico *A. auricula-judae*; en el segundo clado *A. fuscosuccinea*; y en el tercer clado *A. polytricha*.

Palabras clave. *Auricularia*, taxonomía, filogenia, Colombia.

INTRODUCTION

Although *Auricularia* is an easily recognized genus of edible mushrooms with a worldwide distribution, it is difficult to identify specimens to species level because of the morphological variation of the fruit-bodies due to characteristics such as color, size,

and hymenial surface which vary with temperature, humidity, sun light and position of the basidiocarp on the substrate (Kobayasi, 1981).

The genus described by Lowy (1952) based on the characteristics of nine zones of the fruit body tissue (Fig. 1), included ten species grouped in two types according to the presence or absence of a medullary layer. Type one, with a medullary layer included *A. cornea*, *A. fuscosuccinea*, *A. tenuis*, *A. emini* and, *A. polytricha* and type two without a medullary layer included *A. auricula-judae*, *A. delicata*, *A. mesenterica*, *A. ornata*, and *A. peltata*. Moreover, the width of the medulla was considered at species level. Kobayasi (1981) included five new species (*A. minor*, *A. eximia*, *A. papyracea*, *A. incrassate*, and *A. hispida*) and several strains of *A. auricula-judae*, *A. polytricha* and, *A. delicata*, based on the morphological characteristics of the fruit bodies, tissue structure, the hairs on the upper surface, and the color of the hymenophore in fresh material as useful characteristics. Bandoni (1984) proposed an alternative classification of Tremellales and Auriculariales based on the characteristics of the basidia, the haploid stage of mycelia and the septal pore apparatus; however

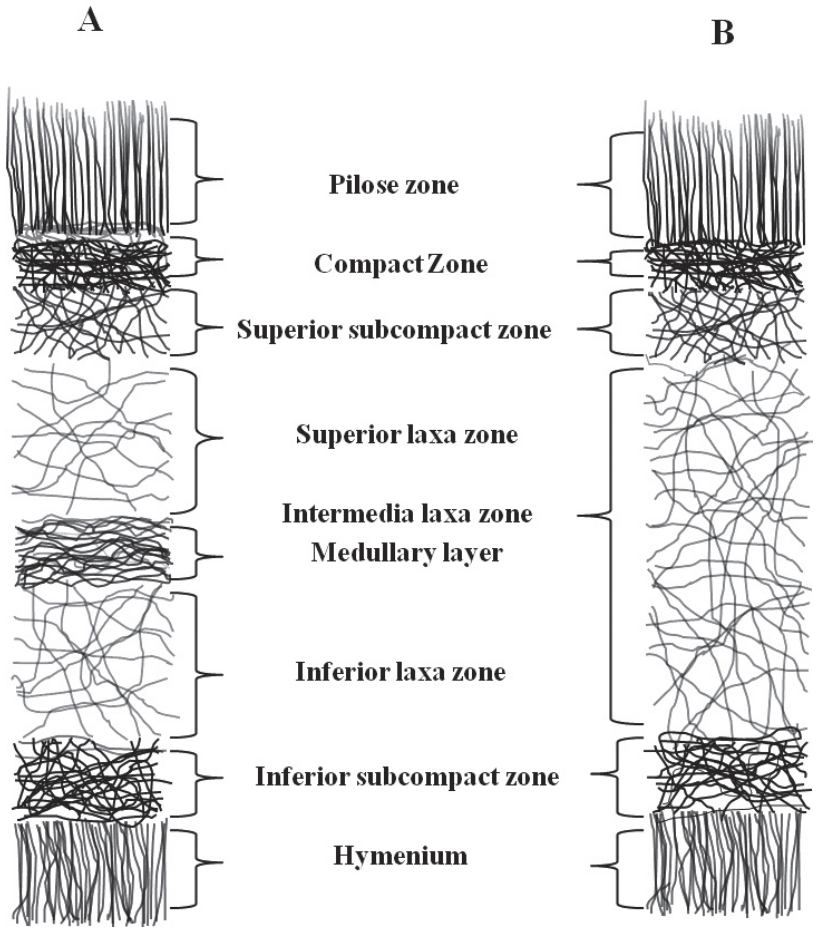

Figure 1. Diagrammatic representation of the hyphal zonation of the basidiocarps types in *Auricularia*. A. Medullated type. B. Nonmedullated type

theses characteristics have not been used to differentiate species. Recent molecular phylogenetic studies revealed new aspects of the relationship between *Auricularia* and allied taxa. WeiB and Oberwinkler (2001) investigated the phylogenetic relationships in Auriculariales and found that *Auricularia* is grouped with *Exidia*, *Exidiopsis*, and *Heterochaete*. However, the number of *Auricularia* species included in the analysis was low, and the phylogenetic relationships within the genus remains unclear. The aim of this study is to investigate the phylogenetic relationships among the species of the genus *Auricularia* in Colombia.

MATERIALS AND METHODS

Seventy-six specimens deposited at the Herbarium of the University of Antioquia (HUA), The National Herbarium of Colombia (COL), and the Herbarium of the University of Quindío (HUQ) and 10 collections gathered during field work in Caldas and Tolima departments of Colombia were examined. The collected specimens were described when fresh and then dried in a food dehydrator (SIGG Dorrex) at 60 °C. Light microscopy studies, were performed in the laboratory of taxonomy and ecology of fungi (TEHO), Institute of Biology, University of Antioquia.

Perpendicular sections were rehydrated in alcohol and water and mounted in H_2O, KOH (5%) and Congo red in order to make microscopic observations and measurements of all zones (pilosa, compact, superior subcompact, intermedia laxa, medulla, inferior subcompact and hymenial) using a calibrated micrometer. For species identification the taxonomic keys of Lowy (1952) and Kobayasi (1981) were used. To understand the phylogenetic relationships within the genus, 17 sequences of *Auricularia*, including 8 previously published sequences, plus two outgroup taxa were analyzed. *Exidiopsis calcea* (AF291280) and *Exidia truncata* (AF291279) from the DNA database of Japan (DDBJ) were used as outgroups. The DNA for phylogenetic analysis was obtained from 300 mg of each *Auricularia* specimen with a Plant Genomic DNA Mini Kit (VIOGENE) used according to the manufacturers' protocols. The isolated DNA was resuspended in TE and stored at -20°C until use. The amplification of the internal transcribed spacer region (ITS) of the nuclear DNA was performed with the following new primers: Mont-ITSF: 5'-CAC ACC TG (T/A) GCA C(C/A)(T/A) TTT CG-3', and Mont-ITSR: 5'-CCG CT(A/G) AAG AGG CC(T/C) A (A/G)G GC-3'. Double-stranded DNA was amplified after incubation at 94°C for final extension at 72°C for 15 min. DNA was amplified by PCR in a 50μl reaction volume containing approximately 50 ng total DNA, 10 mM Tris-HCL buffer (pH 8.3) with 50 Mm KCL and 1.5 $MgCl_2$, 0.2 mM of each dNTP, 1.25 units *Taq* DNA polymerase (TAKARA), and 0.5 μM of each primer. After amplification, reaction mixtures were subjected to electrophoresis in 1% low-melting-temperature agarose gels to purify amplicons. We sequenced the purified PCR products using a BigDye Terminator ver. 3.1 (Applied BioSystems) and ABI Prism 3100 Genetic Analyzer (Applied BioSystems) according to the manufacturer's instructions. For sequencing, we used the same primers as those used for amplification. All sequences have been deposited in DDBJ/EMBL/GenBank International DNA databases (Table 1).

Data analyses

To construct a phylogenetic tree based on ITS sequences of *Auricularia*, sequences were assembled and manually examined for errors using FinchTV software and the amplified regions (minus the length of the primers) were aligned using CLUSTAL W (Thompson et al., 1994) in MEGA version 5 (Tamura et al., 2011) with default settings.

Table 1. list of specimens used in molecular phylogenetic analyses.

Number	Species	Collection no.	Locality	Gen Bank accession (ITS)	References
1	*Auricularia polytricha*	AFM 22	Tsushima, Japan	AB615227	(This study)
2			Jiangsu, China	FJ792587	Jiang, J. et al. (2009) Unpublished
3			Beijing, China	FJ617297	Du, P. (Unpublished)
4		L 523	Antioquia, Colombia	AB615228	(This study)
5			Beijing, China	FJ617301	Du, P. (Unpublished)
6	*Auricularia fuscosuccinea*	LFV 326	Antioquia, Colombia	AB615229	(This study)
7		AFM 13	Caldas, Colombia	AB615230	(This study)
8		FP 917	Antioquia, Colombia	AB615231	(This study)
9		MW 530	Costa Rica	AF291270	WeiB and Oberwinkler (2001)
10	*Auricularia delicata*	USJ 54470	Costa Rica	AF291269	WeiB and Oberwinkler (2001)
11	*Auricularia auricula-judae*	MW 446	Germany	AF291268	WeiB and Oberwinkler (2001)
12			Tsushima, Japan	AB615232	(This study)
13			Xiang Fang, China	GQ168503	Quing, S.R. et al. (Unpublished)
14			Shaanxi, China	EU520071	Yu, Z. (Unpublished)
15	*Auricularia mesenterica*	HE 489	Islas Cook, USA	AB615233	(This study)
16	*Exidiopsis calcea*	MW 531	Germany	AF291280	WeiB and Oberwinkler (2001)
17	*Exidia truncata*	MW 365	Germany	AF291279	WeiB and Oberwinkler (2001)

Phylogenetic relationships were analyzed using the neighbor-joining (NJ), Maximum parsimony (MP) and Maximum likelihood (ML) methods combined with a Bootstrap analysis involving 1000 replication rounds. The transition: transversion ratio was fixed at 2:1. Kimura's 2-parameter method was used for the calculation of the genetic distances.

RESULTS

Five species of *Auricularia* (*A. polytricha*, *A. fuscosuccinea*, *A. delicata*, *A. auricula- judae*, and *A. mesenterica*) were identified from the specimens collected in Colombia in field work or preserved in the Herbariums.

Phylogenetic analysis

The ITS sequences were obtained from seven samples of *Auricularia* from Colombia and Japan. The highest BLAST hits in DDBJ/EMBL/GenBank to all *Auricularia* species has > 97 % similarity. The alignment of seven sequences of *Auricularia* adding to eight sequences of *Auricularia* previously published and two outgroups indicate that the length of the ITS1 and ITS2 region varied from 348 to 391 bp. The phylogenetic relationships of the species of *Auricularia* was analyzed using the models of neighbor joining, maximum parsimony and maximum likelihood.

The results of phylogenetic analysis obtained indicate that *Auricularia* is monophyletic. In this group, *A. mesenterica* was positioned at the most basal node. The remaining *Auricularia* samples were into separated in three clades. The first clade consisted of fourth samples of *A. auricula-judae*; the second clade consisted of fourth samples of *A. fuscosuccinea*, and the third clade consisted of five samples of *A. polytricha* (Fig. 3). High bootstrap support was computed for the cluster

of *A. polytricha* and *A. fuscosuccinea*. Also high bootstrap values were achieved for he cluster of *A. auricula-judae*. The phylogenetic relationships of *A. delicata* could not be significantly resolved. With the exception of *A. delicata*, the same groups obtained by neighbor-joining analysis are also present in the tree that was found applying models of maximum likelihood (Fig. 4) and maximum parsimony (not shown).

Taxonomic key

Key for the identification of the species of the genus *Auricularia* present in Colombia

1. Hymenophore reticulate.....*A. delicata*
1. Hymenophore smooth.....2
2. Cross section with medullary layer.....3
2. Cross section without medullary layer..4
3. Pilose zone less than 100 µm, medullary layer less than 150 µm.....*A. fuscosuccinea*
3. Pilose zone more than 100 µm, medullary layer more than 150 µm.....*A. polytricha*
4. Pilose zone less than 150 µm.....*A. auricula-judae*
4. Pilose zone near to 500 µm.....*A. mesenterica*

The genus *Auricularia* (Builliard ex Merat, Nouv. Fl. Env. Paris, ed. 2, 1: 33. 1821.)

Macroscopically, the members of the genus are characterized by having gelatinous, resupinate to substipitate, saprobic, and solitary to gregarious basidiocarps. Basidiocarps are of 6 to 12 cm in diameter and 1-2 mm thick ; the upper (superior) surface is pilose and dark yellow to brown or reddish brown; the lower (inferior) surface smooth, rugulose to meruloid, glabrous to pruinose and concolorous with the upper surface. Microscopically, in tangential section, a hyphal zonation (pilosa, compact, superior subcompact, intermedia laxa, medulla, inferior subcompact and hymenial) is observed. The basidia are cylindrical to clavate, and transversely 3-septate. The basidiospores are inamyloid, transparent and allantoids.

Description of the species

1- *Auricularia auricula-judae* (Bull:Fr.) Schroet, Krypt. Fl. Schles. 3:386. 1888.
Auricularia auricula (Hook.) Underw; Barret, Mycologia 2: 12. 1910

Basidiocarps gelatinous when fresh, yellow to dark brown, superior surface pilose, inferior surface with few folds, 3.5-6 cm wide; substipitate, 1.3-1.7 cm long, cylindrical and solid; pilose zone shows hairs of 30-150 µm long, hyaline, often with broken tips. Compact zone 20-90 µm in width. Superior subcompact zone 20-100 µm wide. Intermedia laxa 120-500 µm wide. Inferior subcompact zone 40-95 µm wide. Hymenium 60-80 µm wide; basidia 40-70 µm. Spores allantoids 16-19 µm long and 4.3-4.7 wide. Generally gregarious on dead wood. Superficially, *A. auricula-judae* resembles *A. fuscosuccinea* in color and texture, but in section it is clearly differentiated by the absence of a medulla in *A. auricula-judae* (Fig. 2). Lowy (1952) and Kobayasi (1981) describe this species with the name *A. auricula*, while other authors (Swann and Taylor, 1993) describe this species as *A. auricula-judae*. We use *A. auricula-judae*, which, according with Dr. R. G. Bandoni (personal communication) is the most accepted name worldwide.

Collections studied

Colombia, Tolima, Murillo, Vereda Las Novillas.2004/4/4. Montoya A. Andrés Felipe. 01 (HUA). Colombia, Caldas, Manizales, Jardín Botánico de la Universidad de Caldas. 2004/2/28. Montoya A. Andrés Felipe. 05 (HUA). Colombia, Caldas, Manizales, Reserva de Rio Blanco, 2004/3/15 Montoya A. Andrés Felipe. 06 (HUA). Colombia, Antioquia, Medellín, Universidad de Antioquia. 2002/5/30.

López-Q. Carlos A. 522 (HUA) Colombia. Antioquia. Porce. 1987/12/3. Saldarriaga Yamile. 315 (HUA). Colombia, Antioquia, Jardín, Vereda Morro Amarillo. 1987/4/24. Velásquez Luis Fernando. 149 (HUA). Colombia, Antioquia, Taraza, Hacienda Las Mercedes. 1988/7/28. Velásquez Luis Fernando. 268 (HUA). Colombia, Antioquia, Urrao, Vereda Quebrada Arriba. 1987/4/5. Pineda Fabio. 230 (HUA), Colombia, Antioquia, San Luis. 1986/7/11. Velásquez Luis Fernando 67 (HUA).

2- *Auricularia delicata* (Fr.) Henn. Engl. Jahr.17: 493.1893.

Laschia delicata (Fr.), Linnaea 5: 553. 1830

Basidiocarps are gelatinous when fresh, flabelliform, orbicular, reniform, yellow to dark brown, superior surface pilose, inferior surface meruloid, reticulate, 4-8 cm; substipitate, 1.0-3.5 cm long, cylindrical and solid; pilose zone covered by hairs 20-180 µm long, hyaline, often blunt or irregularly rounded tips. Compact zone 20-110 µm wide. Superior subcompact zone 40-270 µm wide. Intermedia laxa zone 60-700 µm wide. Inferior subcompact zone 35-270 µm wide. Hymenium 40-180 µm wide; basidia 30-70 µm. Spores allantoid 9.4-12.3 µm long and 4.3 wide (Fig. 2). Generally gregarious, on wood of species of the genera *Alnus* and *Quercus.*

Collections studied

Colombia, Risaralda, Pueblo Rico, Reserva Karagabi. 2004/6/23. Montoya A. Andrés Felipe. 02 (HUA). Colombia, Risaralda, Pueblo Rico, Reserva Karagabi. 2004/6/23. Montoya A. Andrés Felipe. 03 (HUA). Colombia. Caldas, Manizales, Reserva Río Blanco. 2004/2/28. Montoya A. Andrés Felipe 04 (HUA). Colombia, Quindío, Quimbaya, Finca El Ocaso. Ayala G. 26 (HUQ). Colombia, Quindío, Quimbaya, Finca El Ocaso. Cañón H. Al. 155 (HUQ).

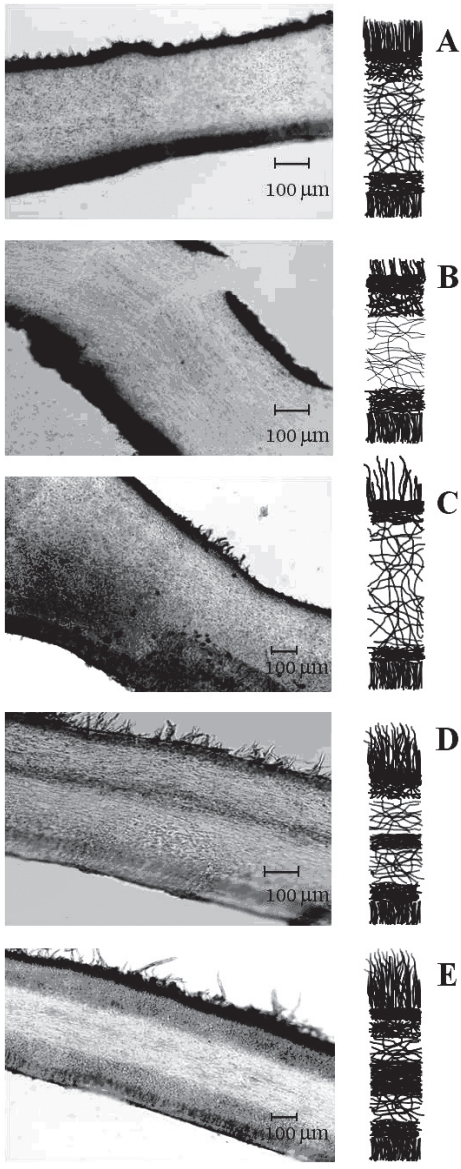

Figure 2. Cross-section of the species of *Auricularia* (left) and diagrammatic representation of the hyphal zone (right). A, B and C are the species without medulla; D and E species with medulla. A. Cross-section of *A. auricula-judae.* B. Cross-section of *A. delicata*. C. Cross-section of *A. mesenterica*. D. Cross-section of *A. fuscosuccinea.* E. Cross-section of *A. polytricha*.

Colombia, Quindío, Quimbaya, Finca El Ocaso. Castaño G. 127 (HUQ). Colombia, Antioquia, Medellín, Vereda Media Luna, Cuenca de la Quebrada Santa Elena. Gallo D. (TEHO). Colombia, Quindío, Quimbaya, Finca El Ocaso. Ayala G. 26 (HUQ). Colombia, Antioquia, Barbosa, Vereda La Cejita. 2000/5/12. Botero Robinson. 07 (HUA) México, Veracruz, Coatepec. 1984/6/8. Chacón S. 2184 (HUA). Colombia, Antioquia, Urrao. 1987/3/29. Pineda Fabio. 215 (HUA). Colombia, Risaralda, Pueblo Rico. 2004/6/23. Montoya A. Andrés Felipe. 7 (HUA). Colombia, Risaralda, Pueblo Rico. 2004/6/23. Montoya A. Andrés Felipe. 8 (HUA). Colombia, Caldas, Riosucio, Vereda la Antioqueña, Resguardo la Montaña. 2004/7/19. Montoya A. Andrés Felipe. 9 (HUA). Colombia, Caldas, Riosucio, Vereda la Antioqueña, Resguardo la Montaña. 2004/7/19. Montoya A. Andrés Felipe. 12 (HUA). Colombia. Valle, Buenaventura, Carretera Cali-Buenaventura. 1986/7/11. Guzman G. 4525 (COL). Leg. Ruby R. Little. 10070 (COL). Leg. Ruby R. Little. 10004 (COL).Colombia, Caquetá, Florencia, Comisaria del Caquetá. E. Pérez Arbeláez. 1032 (COL). Colombia, Cundinamarca, Medina, Cercanías del Boquerón de Santa Inés. Pinto P. 1595 (COL). Colombia, Huila, La Plata, Vereda Agua Bonita, Finca Merenberg. Santiago Díaz. 614 (COL). Colombia, Cundinamarca, Granada, Hacienda el Soche. K.P. Dumont. 1881 (COL). Colombia, Caquetá, Florencia Comisaria del Caquetá. Enrique Pérez Arbeláez. 1032ª (COL). Colombia, Caquetá, Solano. 2004/8/4. Vasco-P. Aida M. 541 (HUA). Colombia, Caquetá, Solano. 2004/8/4. Vasco-P. Aida M. 133 (HUA). Colombia, Caquetá, Solano.2004/8/4. Vasco-P. Aida M. 143 (HUA).All the specimens studied showed a high variation when compared with those reported in the literature, however all specimens had a reticulate-meruloid hymenophore.

3- *Auricularia fuscosuccinea* (Mont.) Farlow, Bibl. Index 1: 307. 1905.

Basidiocarps are gelatinous when fresh, coriaceous when dry; orbicular, reniform, yellow traslucent to dark brown, superior surface pilose, inferior surface smooth with folds, 4.6-10.3 cm; substipitate, 1.8-3.2 cm long, cylindrical and solid; pilose zone with 20-90 µm long, hyaline hairs. Compact zone 20-60 µm wide. Superior subcompact zone 10-50 µm wide. Superior laxa zone 80-200 µm wide. Medullary layer 30-110. Inferior subcompact zone 35-300 µm wide. Inferior laxa zone 40-90µm. Hymenium 70-90 µm wide; basidia 30-50 µm. spores allantoids 8-10.8 µm long and 4.2-6.5µm wide (Fig. 2). Solitary or gregarious, on wood of *Quercus sp*.

Collections studied

Colombia, Quindío, Quimbaya, Finca el Ocaso. Cañón H. 100 (HUQ). Colombia, Quindío, Quimbaya, Finca el Ocaso. Castaño G. 202 (HUQ). Colombia, Quindío, Quimbaya, Finca el Ocaso. Castaño G. 089 (HUQ). Colombia, Caquetá, Solano, Corregimiento Araracuara. 1999/4/3. Álvarez. 134 (TEHO). Colombia, Antioquia, San Luis. 1986/ 7/11. Saldarriaga Yamile 75 (HUA). Colombia, Antioquia, Taraza, Hacienda las Mercedes. 1988/7/27. Pineda Fabio. 405 (HUA). Colombia, Antioquia, Jardín. 1991/17/5. Betancur Adriana. 50 (HUA). Colombia, Antioquia, Jardín. 1989/6/2. Pineda Fabio. 500 (HUA). Colombia, Antioquia, Medellín, Reserva Rio Claro.1987/8/19. Saldarriaga Yamile. 257 (HUA). Colombia, Antioquia. Jardín.1992/11/28. Saldarriaga Yamile. 676 (HUA). Colombia, Antioquia, Medellín, Reserva Rio Claro. 1988/8/31. Velásquez Luis Fernando. 326 (HUA). Colombia, Antioquia, Medellín, Reserva Río Claro. Quijano Andrea. (HUA). Colombia, Antioquia, Medellín, Reserva Río Claro.

García Gustavo. 121 (HUA). Colombia, Antioquia, Santo Domingo. 1994/7/10. Pineda Nicolás. 865 (HUA). Colombia, Antioquia. San Luis. 1987/11/3. García Gustavo. 136 (HUA). Colombia, Antioquia, Puerto Triunfo, Cueva del Cóndor. 1994/3/26. López John Jairo.762 (HUA). Colombia, Antioquia, Santo Domingo, Corregimiento de Porce. 1993/10/1. Pineda Fabio. 771 (HUA). Colombia, Antioquia, Medellín, Reserva Río Claro. 1996/11/23. Saldarriaga Yamile. 917 (HUA). Colombia, Antioquia. San Luis. 1987/11/12. García Gustavo. 141 (HUA). Colombia, Cauca, Popayán. Dumont K.P. 1265 (COL). Colombia, Antioquia, Anori, Rio Anori. K.P.Dumont. 662 (COL). Colombia, Córdoba, Tierra Alta, Quebrada del espíritu santo, Laguna de la Raya. Uribe Fernando. (COL). Colombia, Antioquia, Anori, Aljibe. K.P: Dumont.776 (COL). Colombia, Antioquia, Anori. K. P. Dumont. 595 (COL). Colombia, Amazonas, Araracuara, La Nevera, Carretera a Puerto Arturo. Galeano G. 1291 (COL). Colombia, Norte de Santander. Sarare. Cuatrecasas L. 12905 (COL). Colombia, Cundinamarca, Mun. La Mesa. Margarita Pulido. 724 (COL). Colombia, Cundinamarca, San Cayetano. Antoine M.Cleef. 6607 (COL). Mun. El Tambo.Depto. Cauca. K.P. Dumont. 1389 (COL). Colombia, Amazonas, Araracuara. L.E.Aguirre. 983 (COL). Colombia, Amazonas, Araracuara.2003/8/3. López-Q. Carlos A. 614 (HUA). Colombia, Amazonas, Araracuara. 2004/8/4. Vasco-P. Aida M. 416 (HUA). Colombia, Amazonas, Araracuara. 2004/8/4. Vasco-P. Aida M. 417 (HUA). Colombia, Amazonas, Araracuara. 2004/8/4. Vasco-P. Aida M. 49 (HUA). Colombia, Caquetá, Solano. 2001/6/24. Vasco-P. Aida M. 326 (HUA). Colombia, Caquetá, Solano. 2001/5/14. Vasco-P. Aida M. 169 (HUA).

4- *Auricularia polytricha* (Mont.) Sacc. Tai R. Instit. Veneto Vi 3: 722.1885.

Hirneola polytricha (Mont.) Fries, K. Vet.-Akad. Handl. 1848: 146. 1849.

Basidiocarps are rubbery, gelatinous when fresh, frequently with a convex dorsal surface, dark brown to dark lilac, upper surface densely pilose, lower surface smooth, 3.5-8 cm; substipitate, 0.5-2.0 cm long, cylindrical and solid; pilose zone with 115-550 µm long, hyaline to yellow traslucent hairs. Compact zone 30-80 µm wide. Superior subcompact zone 80-270 µm wide. Superior laxa zone 80-260 µm wide. Medullary layer broad 60-250. Inferior subcompact 35-300 µm wide. Inferior laxa zone 40-90µm. Hymenium 60-80 µm wide; basidia 40-70 µm. Spores were not found in the studied collections (Fig. 2).

Collections studied

Colombia, Quindío, Quimbaya, Finca el Ocaso. Cañón H. 156 (HUQ). Colombia, Antioquia, San Luis. 1986/7/11. Velásquez Luis Fernando 63 (HUA). Colombia, Antioquia, San Luis, Vereda Jerusalén. 1986/7/11 Guzmán G. 29223 (HUA). Colombia, Antioquia, Jardín. 1993/7/8. Pineda Fabio 731 (HUA). Colombia, Choco, Acandi. 1989/12/26. Marco A Correa 01 (HUA). Colombia, Antioquia, Santo Domingo, Vereda Puente Galeano. 1994/10/7. Saldarriaga Yamile 825 (HUA). Leg. Ruby R. Little. 10071 (COL). Colombia, Choco, Riosucio, Cacarica. Horacio Echeverri. 17(COL). Colombia, Choco, Riosucio, Cacarica. Henry León. 17 (COL). Colombia, Cundinamarca, Tocaima, Hacienda el Cucharo. E.P. Killip. 38360 (COL). Brazil, Sao Paulo. Pinto Viegas 79 (COL). Colombia, Antioquia, Medellín, Universidad de Antioquia. 2002/30/5. López-Q. Carlos A. 523 (HUA). Colombia, Caldas, Riosucio, Vereda la Antioqueña, Resguardo la Montaña. 2007/10/30. Montoya A. Andrés Felipe. 13 (HUA).

6- *Auricularia mesenterica* Pers. Myc. Eur. 1 : 97. 1822.

Auricularia lobata (Sommerf.) Mag. For Naturvindesk 7: 296. 1826

Basidiocarps are rubbery when fresh, resupinate, commonly lobed, dark brown to dark lilac, superior surface concentrically zonate, densely pilose, with few folds, inferior surface smooth, 3-7 cm wide; cylindrical and solid; pilose zone with 120-560 µm long, hyaline to yellow translucent hairs. Compact zone 32-46 µm wide. Superior subcompact 140-160 µm wide. Intermedia laxa zone 320-580 µm wide. Inferior subcompact zone138-152 µm wide. Hymenium 68-120 µm wide; basidia 32-58 µm. Spores were not found in the studied collections (Fig. 2).

Collections studied

Colombia, Valle, Sarzal, Carretera Cali-Sarzal. Guzmán G. 4584 (COL). Colombia, Choco, Riosucio, Cacarica, Parque Nacional Natural Los Katios. Horacio Echeverri. 032 (HUA). U.S.A. Islas Cook. H.E. et S.T.Parks 489. Ex USA71310 (COL).

DISCUSSION

We have studied five species of *Auricularia* from Colombia. The most common species are *A. fuscosuccinea* and *A. delicata*, while *A. mesenterica* are the scarcest. Only three specimens of *A. mesenterica* were studied from the herbarium samples. Our results support the monophyletic origin of the genus *Auricularia. A. mesenterica* is positioned in the most basal clade, relatively close to outgroups species

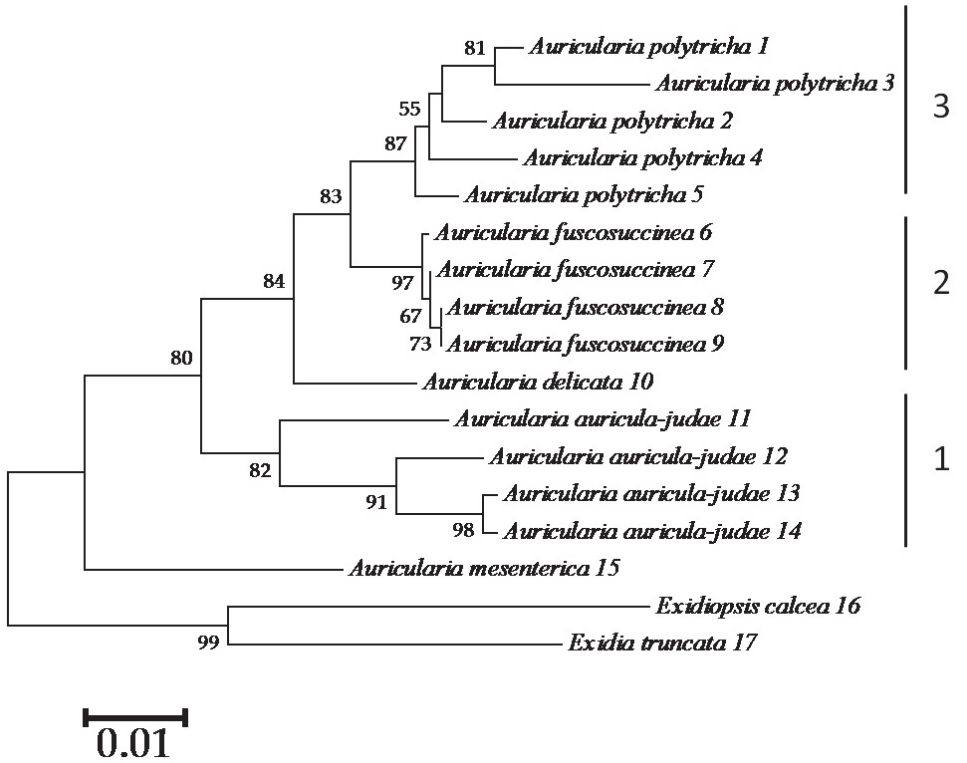

Figure 3. Neighbor-joining analysis of an alignment of nuclear DNA coding. Bootstrap support (> 50%) are shown in each node. The tree is rooted with *Exidiopsis calcea* and *Exidia truncata.*

Exidiopsis calcea and *Exidia truncata*. *A. mesenterica* is the only resupinate specie in the genus *Auricularia* and is similar to the species of *Exidia* and *Exidiopsis*. WeiB and Oberwinkler (2001) reported high similarity between species of *Exidia*, *Exidiopsis* and *Auricularia* using the 28 S ribosomal large subunit and, those results were considered evidence in agreement with Bandoni`s hypothesis (1984), that the basidium of *Auricularia* species is directly derived from that of the exidioid fungi. *A. auricula-judae* is a monophyletic taxon positioned relatively close to *A. mesenterica* and although *A. auricula-judae* is sessile to substipitate, it differs from *A. mesenterica* by being resupinate. These two species both lack a medulla and they could be differentiated in section by differences in the sizes of the pilosa zone and laxa intermedia zone.

WeiB and Oberwinkler (2001) previously reported that *A. delicata* and *A. fuscosuccinea* occurred together in the same cluster supported by a high bootstrap values obtained by neighbor-joining analysis. However, although in our studies, *A.delicata* is positioned near to *A. fuscosuccinea* in the dendrogram obtained by neighbor-joining analysis (Fig.3) and maximum parsimony (not shown); whereas in the maximum likelihood analysis (Fig.4), *A. delicata* was positioned relatively close to *A. auricula-judae*. Although *A. delicata* is a species that lacks medulla; this is a taxon with a characteristic unique in the genus and that is the meruloid form of the inferior surface of the basidiocarps. In this study, the phylogenetic relationship of *A. delicata* could not be significantly resolved.

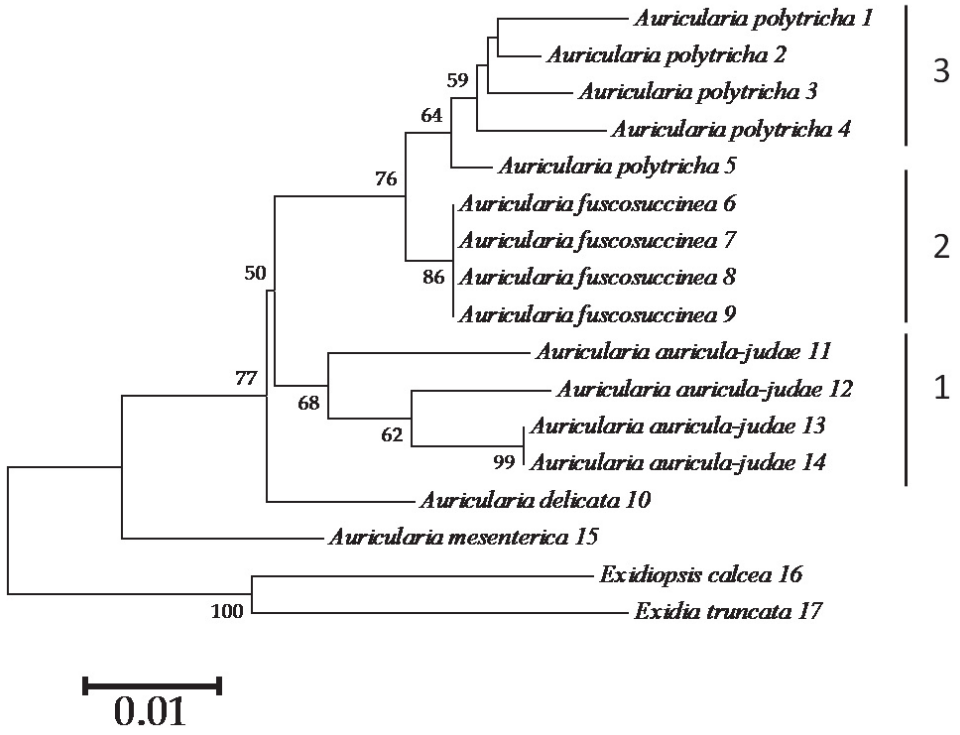

Figure 4. Maximum likelihood analysis of an alignment of nuclear DNA coding. Bootstrap support (> 50%) are shown in each node. The tree is rooted with *Exidiopsis calcea* and *Exidia truncata.*

A. fuscosuccinea is monophyletic supported by high bootstrap values and was positioned in the same cluster with *A. polytricha*. Both *A. fuscosuccinea* and *A. polytricha* are species with medulla that can be differentiated by thickness of the pilosa zone and medullar layer.

Mengyao *et al*., (2008) reported molecular diversity of *A. polytricha*. They found 5 groups from 19 samples of *A. polytricha* and reported that the different *A. polytricha* strains not only vary in their output, cultivation cycle, shape and taste, but also in pharmacological effects. However, the description of *A. polytricha* by Kobayasi (1981) differs substantially from the description by Lowy (1952), mainly in the size of the medullary layer and the length of the pilose zone. In our observations more variance occurred in the width of the hyphal zonations than that is reported in previously published accounts (Lowy, 1952; Kobayasi, 1981). Also, our studies suggest that in the genus *Auricularia* and mainly in *A. polytricha* and *A. auricula-judae* there could be a lot more geographical variation within these taxa than has been recognized by mycologist. (Fig. 3)

The phylogenetic tree based on neighbor-joining analysis clearly shows that in *Auricularia* the absence or presence of medulla is an important characteristic in the identification of species. It is evident that *A. fucosuccinea* and *A. polytricha* are sister groups of species with a medulla. However, further studies are necessary to clarify the relationships between *A. delicata* and others species of the genus and the probably geographic variation between species.

ACKNOWLEDGEMENTS

The authors wish to express their gratitude to the Herbarium of the University of Antioquia (HUA), The National Herbarium of Colombia (COL), and the Herbarium of the University of Quindío (HUQ) for the loan of specimens. The first author would like to thank to Dennis Murphy for comments and correcting English, and to the staff of the Laboratory of Silviculture, Faculty of Agriculture, Kochi University for their invaluable help and assistance in the molecular analysis. We also thank to Dr. Ryo Arakawa for their support throughout this study.

LITERATURE CITED

Bandoni, R.J. 1984. The Tremellales and Auriculariales: an alternative classification. Transactions of the Mycological Society of Japan 25: 489-530.

Kobayasi, Y. 1981. The genus *Auricularia*, *Bull. Natl. Sci. Mus. Tokyo B*, 7: 41-67.

Lowy, B. 1952. The Genus Auricularia. Mycologia. 44: 656-693.

Mengyao Y., M. Bo, L. Xia, Z. Linyong, X. Xiaoyan & Y. Zhirong. 2008. Molecular diversity of *Auricularia polytricha* revealed by inter-simple sequence-related amplified polymorphism markers. Current microbiology: 56 (3): 240-245.

Swann, E.C. & J.W. Taylor. 1993. Higher taxa of basidiomycetes: An 18s RNA gene perspective. Mycologia 85: 923-936.

Swofford, D. L. 2001. *PAUP**. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei & S. Kumar. 2011. MEGA5: Molecular evolutionary genetics analysis using likelihood, distance, and parsimony methods. Molecular biology and evolution.

Thompson, J. D., D.G. Higgins & T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence

weighting, positions-specific gap penalties and weight matrix choice. *Nuc. Ac. Res.* 22, 4673-4680.

WEIB, M. & F. OBERWINKLER. 2001. Phylogenetic relationships in Auriculariales and related groups - hypotheses derived from nuclear ribosomal DNA sequences, *Mycol. Res.*105: 403-415.

Recibido: 24/03/2010
Aceptado: 12/03/2011